ISSN 1346-7565 Acta Phytotax. Geobot. 61 (1): 1–20 (2010)

Phylogeography of *Carpinus japonica* and *Carpinus tschonoskii* (Betulaceae) Growing in Japanese Deciduous Broad-leaved Forests, Based on Chloroplast DNA Variation

TAKAYA IWASAKI[1*], AKITAKA TONO[1], KYOKO AOKI[2], AKIHIRO SEO[3] AND NORIAKI MURAKAMI[1]

[1]*Tokyo Metropolitan University, Graduate School of Science and Engineering, Minami-Osawa 1-1, Hachioji, Tokyo 192-0397, Japan. *takaiwa@center.tmu.ac.jp (author for correspondence);*
[2]*Kyoto University, Graduate School of Global Environment Studies, Sakyo-ku, Kyoto 606-8501, Japan;*
[3]*Research Institute for Humanity and Nature, Kamigamomotoyama 457-4, Kita-ku, Kyoto 603-8047, Japan*

A phylogeographic study of two species of *Carpinus*, *C. japonica* Blume and *C. tschonoskii* Maxim. (Betulaceae), based on the distribution patterns of their chloroplast DNA haplotypes, is reported. In Japan, these species are mainly distributed in Pacific-type deciduous broad-leaved forests. Using 439–440 and 627–629 bp nucleotide sequences of noncoding regions of chloroplast DNA, we detected 5 and 6 haplotypes among 217 and 181 individuals sampled from 52 and 49 populations of *C. japonica* and *C. tschonoskii*, respectively. The geographic distribution patterns of the haplotypes were highly structured. We investigated the common phylogeographic patterns between the two species that would indicate the influence of common historical factors such as climate change since the last glacial maximum (LGM). Based on our results, we concluded that the Pacific-type Japanese deciduous broad-leaved forests were split into at least three refugia during the LGM. After the LGM, the species expanded to northern areas or moved to higher altitudes from each refugium, thus now occupying northeastern, central, and southwestern Japan.

Key words: *Carpinus*, chloroplast DNA, glacial refugia, intraspecific variation, Japanese archipelago, Pacific-type deciduous broad-leaved forest, phylogeography

Repeated severe climatic changes during the Quaternary period produced great changes in species distributions (Hewitt 2000, 2004). During the last glacial maximum (LGM) around 18,000–23,000 years ago, the mean annual temperature was 5–9°C cooler and the precipitation was much less in comparison with the climate in Japan at present (Tsukada 1988). The historical changes in vegetation in Japan since the LGM have been studied in detail over the last few decades (Tsukada 1988, Yasuda & Miyoshi 1998, Takahara *et al.* 2000). In response to climatic change, temperate plant species in Japan have generally migrated along the Pacific Ocean side, Sea of Japan side, or along mountain slopes of the archipelago, i.e., migrating either southward along the coasts or to lower altitudes into refugia during glacial periods and expanding either northward or to higher altitudes during interglacial periods (Tsukada 1988). The temperate zone in Japan is now dominated by deciduous broad-leaved tree species, primarily *Fagus crenata* and *Quercus crispula*. Deciduous broad-leaved forests in northeastern Japan occur from sea level to an altitude of more than 1000 m. In contrast, those in southwestern Japan are often isolated

from each other in small patches at elevations over 700 m. In addition, the Japanese deciduous broad-leaved forests can be divided into two types, the Pacific-type and the Sea of Japan-type, on the basis of ordination by both factor and principal component analyses using the frequency of tree species in each type of forests (Fujita 1987). The Sea of Japan side of this archipelago is characterized by heavy snowfall in winter while the Pacific Ocean side has a milder and drier climate in winter. The component species of each forest type are considered to have adapted to their different environments (Fujita 1987). The migration histories of the component species of these two types of forest after the LGM might also be different, but the colonization history of species of trees in the two forest types has not been well investigated. Although fossil pollen was utilized to determine the constituent genera, e.g., *Carpinus* or *Fagus* (Tsukada 1988, Yasuda & Miyoshi 1998), it is often difficult to identify species based only on the morphological characteristics of pollen.

Recent molecular phylogeographic studies have revealed the genetic structure of extant populations of organisms, and also provided insight into the history of species distribution changes (Avise 2000). In general, the response of species to climatic oscillations is considered a highly dynamic process, consisting of repeated retreats into refugia during the glacial period and interglacial range expansions from the refugia. Because chloroplast DNA (cpDNA) is predominantly maternally inherited in angiosperms (Corriveau & Coleman 1988) without any recombination, the maternal lineage can be traced at the level of whole chloroplast genome. Thus, cpDNA is extremely useful for determining the route of seed migration and identifying locations of important refugia during the LGM (McCauly 1995, Newton *et al.* 1999, Abbott *et al.* 2000, Heuertz *et al.* 2004). Over the past decade, the geographic distribution of intraspecific cpDNA variation has been examined for various species of Japanese deciduous trees, including *Fagus crenata* (Fujii *et al.* 2002, Okaura & Harada 2002), *Stachyurus praecox* (Ohi *et al.* 2003), *Quercus crispula* and related species (Okaura *et al.* 2007), to elucidate the history of postglacial recolonization. Those species are widely distributed in Japanese deciduous broad-leaved forests, but are not major components of Pacific type forests. No phylogeographic studies using intraspecific cpDNA variation have been conducted for major component species of the Pacific-type forests. *Fagus japonica*, which is distributed mainly in Pacific-type forests, is one of the rare examples whose intraspecific genetic structure has been investigated, although it was not analyzed using cpDNA variation but by using 13 nuclear microsatellite markers (Hiraoka & Tomaru 2009). It has been reported that *F. japonica* can be divided into three genetic populations based on their occurrence in northeastern, central, and southwestern Japan. Phylogeographic studies on the major components of the Pacific type forests are, however, still insufficient. To elucidate the colonization history of Pacific-type forests in Japan, there is a need to compare the intraspecific genetic structure of different component species of those forests, and to identify common phylogeographic patterns.

In this study, we investigated intraspecific cpDNA variation in *Carpinus japonica* Blume and *C. tschonoskii* Maxim., in which a relatively large amount of intraspecific cpDNA variation was observed (Iwasaki *et al.* 2006), to elucidate common phylogeographic patterns. The two species grow together and are co-distributed in the Japanese deciduous broad-leaved forests from Kyushu to the southern Tohoku region. We also discuss the colonization history of Pacific-type forests based on their common phylogeographic patterns. Species of *Carpinus* (Betulaceae) are major components of Pacific-type Japanese temperate deciduous forests (Fujita 1987, Nozaki & Okutomi 1990). They have wind-pollinated flowers and relatively small wind-dispersed seeds. While *C. japonica* is endemic to Japan, *C. tschonoskii* also occurs in Korea and China (Ohba 2006). They are common in forests on the Pacific Ocean side, but rare in forests on the Sea of Japan side of Japan (Fujita 1987). Three additional species of *Carpinus*, *C. cordata* Blume, *C. laxiflora* (Siebold & Zucc.) Blume, and *C. turczaninovii* Hance, are in

Japan. Two, *C. cordata* and *C. laxiflora*, sometimes co-occur with *C. japonica* and *C. tschonoskii*. Introgression of cpDNA from related species was reported in various plant taxa (Rieseberg & Soltis 1991, Belahbib *et al.* 2001). Such introgression has the potential to confound intraspecific phylogenies (Rieseberg & Soltis 1991) and to complicate geographic patterns of intraspecific variation (Belahbib *et al.* 2001). We therefore also examined the possibility of cytoplasmic exchange among *C. japonica*, *C. tschonoskii*, *C. cordata*, and *C. laxiflora*.

Our specific goals were to investigate the levels and distribution of intraspecific cpDNA variation and phylogeographic patterns in the two species of *Carpinus* and to discuss the migration history of Pacific-type forests after the LGM.

Materials and Methods

Plant materials

Two hundred and seventeen individuals of *Carpinus japonica* from 52 populations, consisting of 1–15 individuals per population, and 181 individuals of *C. tschonoskii* from 49 populations, consisting of 1–11 individuals per population were collected to cover their entire range in Japan. To determine the possibility of cytoplasmic exchange among the species of *Carpinus*, material from one individual was collected for both *C. cordata* and *C. laxiflora*. Leaves were collected and dried in silica gel. Continental Asian populations of *C. tschonoskii* may influence the phylogeographic pattern of Japanese populations, but we were unable to obtain specimens from Korea and China for this study. Voucher specimens were collected for each site and are preserved in the Makino Herbarium (MAK) of Tokyo Metropolitan University. Geographic and voucher information for the plant samples are shown in Appendix 1. Geographic information for the regions of Japan is shown in Fig. 1.

DNA extraction, polymerase chain reaction (PCR) amplification, and nucleotide sequencing

Prior to genomic DNA extraction, the leaf tissue was washed with 2-[4-(2-hydroxyethyl)-1-piperazininyl] ethanesulfonic acid (HEPES) buffer (pH 8.0) (Setoguchi & Ohba 1995) to remove polysaccharides. Genomic DNAs were extracted from the washed leaf pellets by the cetyltrimethylammonium bromide (CTAB) method (Doyle & Doyle 1987) with slight modification. As a preliminary screening, we analyzed six noncoding regions of cpDNA for each species; the *rps16* intron, *trnG* (UCC) intron, *rpl16* intron, *trnW* (CCA)–*trnP* (UGG) intergenic region (Nishizawa & Watano 2000), *trnL* (UAA) 3′ exon–*trnF* (GAA) intergenic region (Taberlet *et al.* 1991), and *trnH* (GUG)–*psbA* intergenic region (Hamilton 1999). In consideration of the amount of intraspecific sequence variation, we selected two regions for each species (data not shown). We used *trnL* (UAA) 3′exon-*trnF* (GAA) and *trnW* (CCA)–*trnP* (UGG) intergenic regions for the analysis of *Carpinus japonica*, and *trnG* (UCC) and *rpl16* introns for the analysis of *C. tschonoskii*. The PCR mixture (25 μL) consisted of 0.63 units of Blend Taq polymerase (Toyobo, Osaka, Japan), 2.5 μL of 10% Blend Taq Buffer

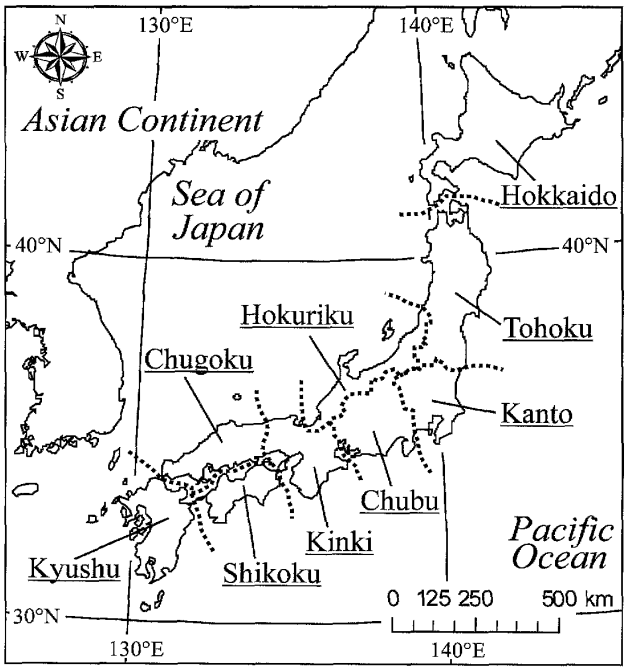

FIG. 1. Location of Japanese archipelago and regions in Japan.

(25 mmol/L TAPS [pH 9.3], 50 mmol/L KCl, and 10 mmol/L $MgCl_2$), 2 μL of 2 mmol/L dNTP solution, 1.25 μL of 10 pmol/L of each primer, and 10–30 ng of genomic DNAs. The PCR cycle conditions were 94°C (2 min); then 35 cycles of 94°C (30 s), 54°C (30 s), 72°C (1 min), and finally 72°C (7 min). After confirming PCR amplification on an agarose gel, the amplified products were treated with ExoSAP-IT reagent (Amersham Biosciences, Tokyo, Japan) to remove excess primers and dNTPs. Purified DNA fragments were used as a template for sequencing reactions using the ABI PRISM Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with the same primers used for PCR amplification. The resultant mixture was analyzed using an ABI model 3100 genetic analyzer (Applied Biosystems). The obtained nucleotide sequences were aligned using the ChromasPro ver. 1.34 software (www.technelysium.com.au/ChromasPro.html) and BioEdit ver. 7.0.9.0 software (Hall 1999).

Intraspecific haplotype network analysis and haplotype diversity

Multiple sequence alignment was performed manually. To examine phylogenetic relationships among the haplotypes observed in each species, intraspecific haplotype networks for the obtained sequence data were inferred using median-joining network analysis based on parsimony criteria (Bandelt *et al.* 1999). The analysis was performed for combined sequence data using the Network 4.5.1 software (Fluxus Technology Ltd. at www.fluxus-engineering.com). Gaps caused by variation in the number of mononucleotide repeat units were removed from the analysis. In addition, to evaluate the amount of intraspecific diversity in each species, haplotype (gene) diversity (*h*) (Nei 1987) was calculated using Arlequin ver. 3.1 software (Excoffier *et al.* 2005).

Interspecific haplotype network analysis among four species of Carpinus

To check for the possibility of cytoplasmic exchanges among the four species of *Carpinus*, we reconstructed phylogenetic relationships among the cpDNA haplotypes observed in the four species. The plant samples used in the analysis are indicated in Appendix 1. Five noncoding regions of cpDNA (*rps16*, *trnG* and *rpl16* introns, and *trnW–trnP* and *trnL–trnF* intergenic regions) were analyzed. For DNA extraction, PCR amplification, and nucleotide sequencing, the same method as mentioned above was used. The interspecific haplotype network for the obtained sequence data was also inferred using median-joining network analysis based on parsimony criteria (Bandelt *et al.* 1999). The analysis was performed for combined sequence data using Network 4.5.1 software (Fluxus Technology Ltd. at www.fluxus-engineering.com). All gaps were removed for the analysis.

Results

Intraspecific cpDNA variation

The nucleotide sequences of cpDNA noncoding regions, including 439–440 bp sequences of *trnL–trnF* and *trnW–trnP* intergenic regions from *Carpinus japonica*, and 627–629 bp sequences of *trnG* and *rpl16* introns from *C. tschonoskii*, were determined. The aligned sequences for polymorphic sites in each species are shown in Tables 1 and 2. The sequence data obtained in this analysis have been deposited in the DNA database of DDBJ (http://www.ddbj.nig.ac.jp) under accession numbers AB532000–AB532009 and AB532010–AB532023 for *C. japonica* and *C. tschonoskii*, respectively. We detected cpDNA variations based on nucleotide substitutions and mononucleotide repeat length variations. The sequences that differed by nucleotide substitutions were treated as different DNA types (indicated by A, B, C, ...). Those differing only by the number of mononucleotide repeats were treated as different subtypes of each DNA type (indicated by A, A′, A″, ...). DNA types denote relative frequency alphabetically, the A-DNA type of each species being the most common. Similarly, the subtypes denote relative frequency, with the A-subtype being the most common, and the A′-subtype being the second most.

In *Carpinus japonica*, 4 DNA types, one of

TABLE 1. Variable sites of the five aligned sequences of chloroplast DNA of *Carpinus japonica*

		Variable positions*			
		trnL–trnF intergenic region (272–273 bp)			*trnW–trnP* intergenic region (167 bp)
Haplotype	Frequency (%)	4	229	257	92
A	36.4	A	T	T_9	C
A′	27.6	A	T	T_8	C
B	34.6	A	T	T_8	T
C	0.9	C	T	T_8	T
D	0.5	A	C	T_9	C

*Mononucleotide repeats are indicated as a nucleotide followed by the number of repeats. Sequences are numbered from the 5′ to the 3′ end in each region.

TABLE 2. Variable sites of the 6 aligned sequences of chloroplast DNA of *Carpinus tschonoskii*

		Variable positions*			
		trnG intron (377–379 bp)			*rpl16* intron (250 bp)
Haplotype	Frequency (%)	4	229	257	92
A	32.0	A	A_{10}	T	T
A′	28.2	A	A_9	T	T
A″	0.6	A	A_{11}	T	T
B	29.8	A	A_9	T	G
C	6.1	A	A_9	C	G
D	3.3	G	A_9	T	G

*Mononucleotide repeats are indicated as a nucleotide followed by the number of repeats. Sequences are numbered from the 5′ to the 3′ end in each region.

which had 2 subtypes, were distinguished, and 5 distinguishable cpDNA haplotypes were recognized in total. Their frequencies were A (36.4%), A′ (27.6%), B (34.6%), C (0.9%), and D (0.5%). In *C. tschonoskii*, 4 DNA types, one of which had 3 subtypes, respectively, were distinguished, and 6 distinguishable cpDNA haplotypes were recognized in total. Their frequencies were A (32.0%), A′ (28.2%), A″ (0.6%), B (29.8%), C (6.1%), and D (3.3%). The phylogenetic relationships among the haplotypes in each species are shown in the parsimony network (Figs. 2 and 3). The values calculated for haplotype diversity were 0.6746 ± 0.0077 in *C. japonica* and 0.7281 ± 0.0120 in C. tschonoskii.

Geographic distribution of haplotypes

The geographic distribution of the cpDNA haplotypes in each species are shown in Figs. 4 and 5. In *Carpinus japonica*, haplotype A was distributed mainly in the Chubu and Kinki regions (population number 4, 5, 13–32, and 35) (Fig. 4). Type A′ was predominant in the Tohoku and Kanto regions (1–12, 18, and 19), and was also disjunctly distributed in the narrow area of southwestern Japan, the Kii Peninsula and the eastern part of Shikoku (35, 41, and 42). Type B was widely distributed in southwestern Japan (12, 26, 29, 33, 34, and 36–52). Two haplotypes, C and D, were found at only one site each; on Mt. Tsukuba (5) (in the southern Kanto region), and on Mt. Aoba (12) (around Wakasa Bay), respectively.

In *Carpinus tschonoskii*, haplotype A occurred in the Kanto, Chubu, and southern part of the Tohoku regions (4–9, and 12–21), and one population in the Kinki region (31) (Fig. 5). Type

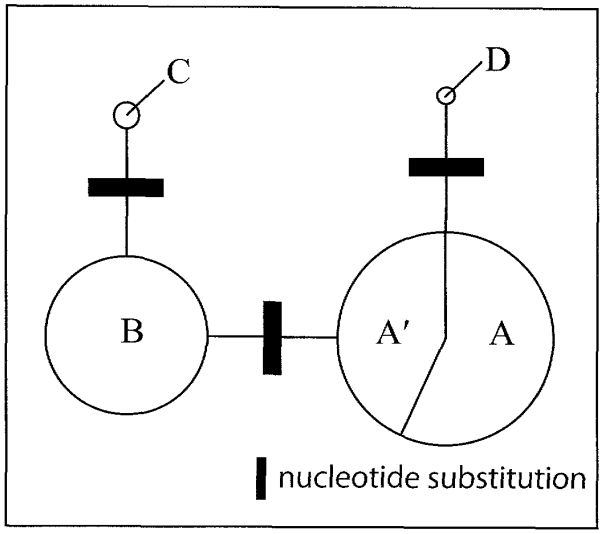

FIG. 2. Median-joining (MJ) network of haplotypes of *Carpinus japonica* using intergenic regions of *trnL–trnF* and *trnW–trnP*. Size of circle is proportional to frequency of haplotype.

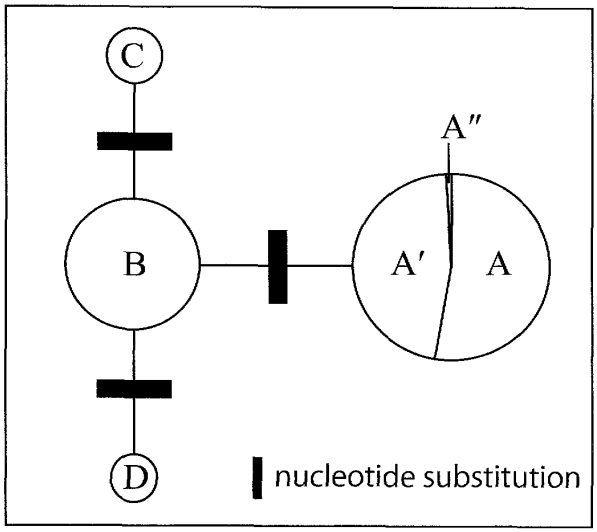

FIG. 3. Median-joining (MJ) network of haplotypes of *Carpinus tschonoskii* using *trnG* and *rpl16* introns. Size of circles is proportional to frequency of haplotype.

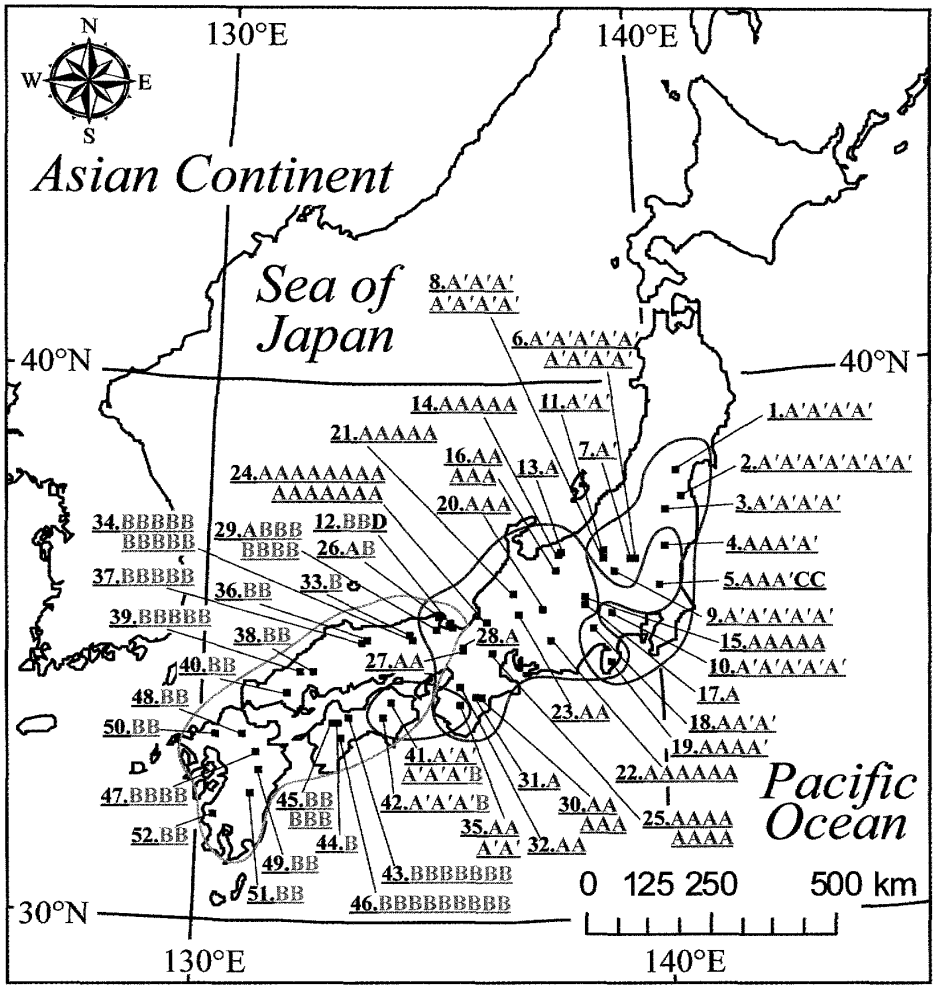

FIG. 4. Geographic distribution of chloroplast DNA (cpDNA) haplotypes in *Carpinus japonica*. Letters with primes indicate a haplotype differing from original haplotype only in number of mononucleotide repeats. Colored letters indicate a common haplotype with a frequency of more than 3%; black letters indicate haplotypes with a frequency of 3% or less. Blue, red, and green circles roughly indicate distribution ranges of three major haplotypes A, A′, and B, respectively.

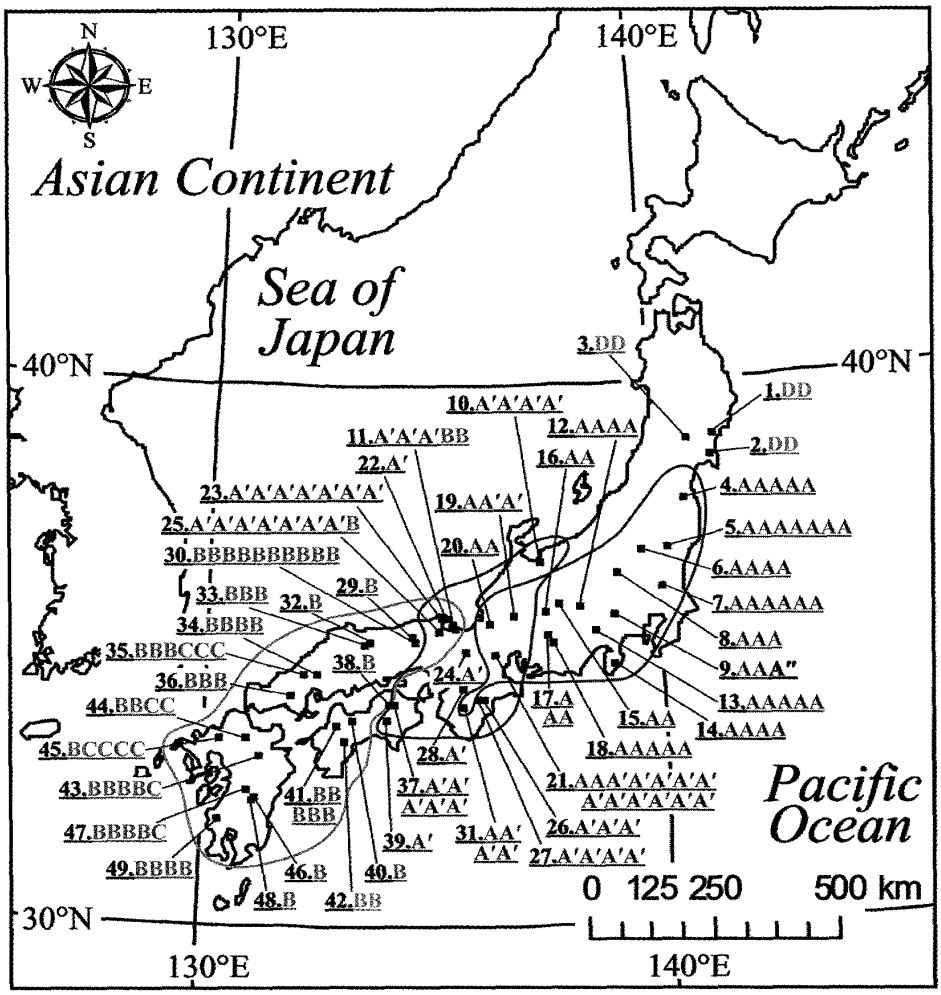

FIG. 5. Geographic distribution of chloroplast DNA (cpDNA) haplotypes in *Carpinus tschonoskii*. Letters with primes indicate a haplotype differing from original haplotype only in number of mononucleotide repeats. Colored letters indicate a common haplotype with a frequency of more than 3%; black letters indicate haplotypes with a frequency of 3% or less. Red, blue, and green circles roughly indicate distribution ranges of three major haplotypes A, A′, and B, respectively.

A′ was predominant in the Kinki region and surrounding areas (Toyama and Gifu prefectures, and the eastern part of Shikoku) (10, 11, 19, 21–28, 31, 37, and 39). Type B was widely distributed in southwestern Japan (11, 25, 29, 30, 32–36, 38, and 40–49). Type C was distributed continuously from the western part of the Chugoku region to the northern part of Kyushu (35, 36, 43–45, and 47). Type D showed local distribution in the Tohoku region (1–3). Type A″ was found only at Sayaguchi Pass (9).

Comparison of haplotype distribution patterns between the two species of Carpinus

The distribution patterns of the cpDNA haplotypes in each species were highly structured geographically (Figs. 4 and 5). Additionally, three major different haplotypes in each species occurred in northeastern Japan, central Japan, and southwestern Japan, respectively. The first haplotype (haplotype A′ in *Carpinus japonica* and type A in *C. tschonoskii*) were in northeastern Japan (roughly corresponding to the Tohoku and Kanto regions in *C. japonica* and in the Tohoku, Kanto and Chubu regions in *C. tschonoskii*). The second haplotype (type A in *C. japonica* and type A′ in *C. tschonoskii*) was in central Japan (roughly corresponding to the Kinki and Chubu regions in *C. japonica* and the Kinki region and surrounding area in *C. tschonoskii*). The third haplotype (type

B in *C. japonica* and type B in *C. tschonoskii*) occurred in southwestern Japan (roughly corresponding to the Chugoku, Shikoku, and Kyushu regions in both *C. japonica* and *C. tschonoskii*). Although the limit of distribution of the haplotypes was unclear, the range of distribution of the haplotypes was similar in the two species of *Carpinus*.

Phylogenetic relationship among the cpDNA haplotypes of the four species of Carpinus

The nucleotide sequences of five cpDNA noncoding regions, a total of 2,000–2,037 bp sequence of *rps16*, *trnG*, and *rpl16* introns, and *trnW–trnP* and *trnL–trnF* intergenic regions, were determined for representative samples of four species of *Carpinus*: *C. japonica*, *C. tschonoskii*, *C. cordata*, and *C. laxiflora*. The sequence data obtained in this analysis have been deposited in the DNA database of DDBJ (http://www.ddbj.nig.ac.jp) under accession numbers AB537987–AB538006 for *C. japonica*, AB538007–AB538026 for *C. tschonoskii*, AB538027–AB538031 for *C. cordata*, and AB538032–AB538036 for *C. laxiflora*. The phylogenetic relationships among the cpDNA haplotypes of the four species of *Carpinus* are shown in the parsimony network (Fig. 6). Most haplotypes of *C. japonica* and *C. tschonoskii* were separately nested together. However, the local haplotype C of *C. japonica* was nested within the haplotype of *C. cordata*. Haplotypes D of *C. tschonoskii* were more closely related to the haplotypes of *C. laxiflora*.

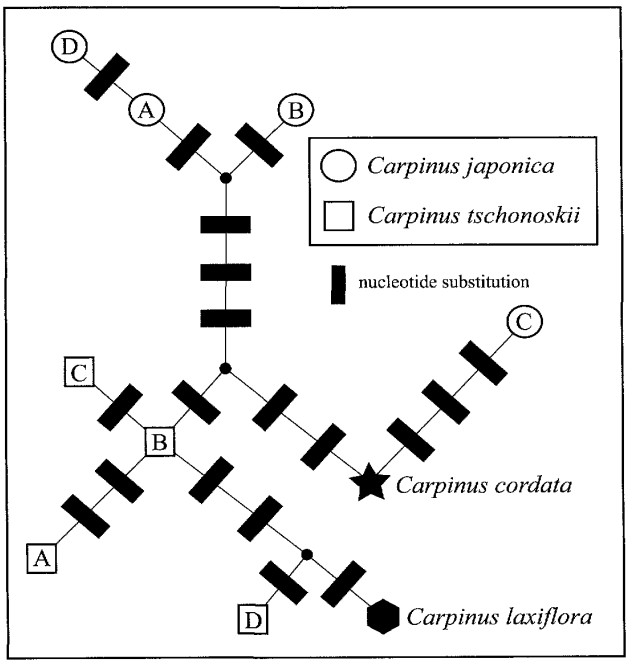

FIG. 6. Median-joining (MJ) network of haplotypes of four species of *Carpinus* (*C. japonica*, *C. tschonoskii*, *C. cordata*, and *C. laxiflora*) using five noncoding regions of chloroplast DNA (*rps16*, *trnG*, and *rpl16* introns, and *trnW–trnP* and *trnL–trnF* intergenic regions).

Discussion

Three phylogeographically distinct areas; the northeastern, central, and southwestern regions of Japan

Three major cpDNA haplotypes in each of two species of *Carpinus*, *C. japonica* and *C. tschonoskii*, occur in three distinctive regions of Japan, northeastern, central, and southwestern regions of Japan (Figs. 4 and 5), according to the results of this study. The distribution patterns of the two species are surprisingly similar and similar to the pattern reported for *Fagus japonica*, using nuclear microsatellite markers, by Hiraoka & Tomaru (2009).

Such similarities in phylogeographic patterns among different tree species may be due to common historical factors (Taberlet *et al.* 1998, Hewitt 2004, Soltis *et al.* 2006). In the two species of *Carpinus*, the three areas were occupied by different widespread cpDNA haplotypes. The most parsimonious interpretation of our observations is that each species was confined to at least three distinct refugia during the LGM, after which the haplotypes expanded to occupy their current range of distribution.

Lack of genetically unique groups of populations on the Sea of Japan side of Japan

Carpinus japonica and *C. tschonoskii* occur mainly along the Pacific Ocean side of Japan and occasionally along the Sea of Japan in the Chubu, Kinki, and Chugoku regions (Figs. 4 and 5). If colonization routes existed after the LGM along the Sea of Japan, some major haplotypes might be expected to occur in that area. In this study, how-

ever, we did not detect any haplotypes that were continuously distributed along the Sea of Japan side. Similarly, in *Fagus japonica*, no unique groups of populations were reported along the Sea of Japan side (Hiraoka & Tomaru 2009). In contrast, previous phylogeographic studies of some component species of Japanese deciduous broad-leaved forests, such as *F. crenata* (Fujii *et al.* 2002), and *Stachyurus praecox* (Ohi *et al.* 2003), found some major haplotypes to be continuously distributed only along the Sea of Japan side. Such discrepancies might be due to different migration histories. The components of the Pacific-type deciduous broad-leaved forests may have expanded not along the Sea of Japan, but expanded from the Pacific Ocean side to the Sea of Japan side after the LGM.

Traces of range expansion during the LGM in southwestern Japan

As mentioned above, southwestern Japan was occupied by one major haplotype in each of the two species of *Carpinus*, suggesting that the colonization history of southwestern Japan was independent from northeastern and central Japan. In the present study, haplotype A′ of *Carpinus japonica* (Fig. 4; population number 35, 41, and 42) was found in the narrow continuous area from the Kii Peninsula to the western part of Shikoku and haplotype C of *C. tschonoskii* (Fig. 5; population number 35, 36, 43–45, 47, and 49) was found in the area from the western part of the Chugoku district to northern Kyushu. Since haplotype A′ of *C. japonica* is disjunct in northeastern Japan, the populations in the narrow continuous area from the Kii Peninsula to the western part of Shikoku could be due to long seed dispersal from the populations in northeastern Japan. Since northeastern Japan and the Kii Peninsula populations are 300 km or more apart, however, it seems more likely that the disjunct populations of haplotype A′ may have originated from independent refugia with the same haplotype.

Two possible explanations are considered to account for local haplotypes shared between nearby populations that are separated by a narrow sea: (1) the populations originated from the same refugium after the LGM, or (2) the populations originated from different refugia, but there is a large amount of gene flow between the populations at present. In southwestern Japan, however, species of *Carpinus* now occur sporadically only on mountains at altitudes of over 700 m, making the latter possibility seem more unlikely. Moreover, during the LGM, due to lower sea levels (ca. 100 m below present), Shikoku and Kyushu were connected to Honshu, and the continental shelf, ca. 20–30 km from the present coastline was above sea level around the Japanese archipelago (Ohta & Yonekura 1987). In addition, palynological data indicate that in southwestern Japan, most of the deciduous broad-leaved trees occurred in coastal areas on the Pacific Ocean and Sea of Japan sides (Kamei & Research Group for the Biogeography from Würm Glacial 1981, Tsukada 1988). As the climate warmed, they moved to higher altitudes (Tsukada 1988, Takahara *et al.* 2000). Therefore, hypothesis (1) seems probable and the following conclusion can be drawn. The sharing of a local haplotype in the narrow continuous area from the Kii Peninsula to western Shikoku and the continuous area from the western Chugoku region to northern Kyushu may be the result of past continuous forests in these areas.

Possibility of local hybridization between different species of Carpinus

In *Carpinus japonica*, we found two individuals with local haplotype C at Mt. Tsukuba (population number 5) (Fig. 4). The haplotype was not similar to other haplotypes of *C. japonica*, but nested within the haplotype of *C. cordata* (Fig. 6). In addition, individuals with haplotype C are morphologically intermediate between *C. cordata* and *C. japonica* (data not shown). Similarly, haplotype D, found locally in three populations of *C. tschonoskii* in the Tohoku regions (Fig. 5), is more similar to the haplotype of *C. laxiflora* (Fig. 6). Those four species of *Carpinus* often grow together in deciduous broad-leaved forests in Japan. Although hybridization between them has not been reported, our results suggest the possibility of hybridization between *C. japonica* and

C. cordata, and between *C. tschonoskii* and *C. laxiflora*.

Conclusion

In this study, we found several phylogeographic patterns that were common to the two Japanese species of *Carpinus* examined (*C. japonica* and *C. tschonoskii*). Additionally, our results agree with those from a previous phylogeographic study of *Fagus japonica* (Hiraoka & Tomaru 2009), which grows with the species of *Carpinus* in Pacific-type Japanese deciduous broad-leaved forests. We therefore theorize that at least three distinct refugia on the Japanese archipelago existed during the LGM. As the climate warmed, the forests expanded to the north or moved to higher altitudes from the three refugia to recolonize northeastern, central, and southwestern Japan.

We thank Dr. Naoki Nishimura, Dr. Tomoko Fukuda, Dr. Wataru Shinohara, Dr. Hirotoshi Sato, Dr. Takashi Sugawara and Dr. Hiroshi Ikeda for their assistance with the collection of plant materials. We also thank Dr. Ikuyo Saeki for valuable advice. This study was partly supported by Research Project 'A new cultural and historical exploration into human-nature relationships in the Japanese archipelago' of the Research Institute for Humanity and Nature and Grant-in-Aid for JSPS Fellows 19-5749 (TI).

References

Abbott, R. J., L. C. Smith, R. I. Milne, R. M. M. Crawford, K. Wolff & J. Balfour. 2000. Molecular Analysis of Plant Migration and Refugia in the Arctic. Science 289: 1343–1346.

Avise, J. C. 2000. Phylogeography: the history and formation of species. Harvard University Press, Cambridge.

Bandelt, H. J., P. Forster & A. Rohl. 1999. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16: 37–48.

Belahbib, N., M. H. Pemonge, A. Ouassou, H. Sbay, A. Kremer & R. J. Petit. 2001. Frequent cytoplasmic exchanges between oak species that are not closely related: *Quercus subur* and *Q. ilex* in Morocco. Mol. Ecol. 10: 2003–2012.

Corriveau, J. L. & A. W. Coleman. 1988. Rapid screening method to detect potential biparental inheritance of plastid DNA and results for over 200 angiosperm species. Am. J. Bot. 75: 1443–1458.

Doyle, J. J. & J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19: 11–15.

Excoffier, L., G. Laval & S. Schneider. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evol. Bioinform. Online. 1: 47–50.

Fujii, N., N. Tomaru, K. Okuyama, T. Koike, T. Mikami & K. Ueda. 2002. Chloroplast DNA phylogeography of *Fagus crenata* (Fagaceae) in Japan. Plant Syst. Evol. 232: 21–33.

Fujita, N. 1987. One-sided distribution of the component species of the Japanese beech forests along either the Pacific or the Japan Sea. Acta Phytotax. Geobot. 38: 311–329. (in Japanese)

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41: 95–98.

Hamilton, M. B. 1999. Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. Mol. Ecol. 8: 521–523.

Heuertz, M., S. Fineschi, M. Anzidei, R. Pastorelli, D. Salvini, L. Paule, N. Frascaria-Lacoste, O. J. Hardy, X. Vekemans & G. G. Vendramin. 2004. Chloroplast DNA variation and postglacial recolonization of common ash (Fraxinus excelsior L.) in Europe. Mol. Ecol. 13: 3437–3452.

Hewitt, G. M. 2000. The genetic legacy of the Quaternary ice ages. Nature 405: 907–913.

Hewitt, G. M. 2004. Genetic consequences of climatic oscillations in the Quaternary. Philos. Trans. Roy. Soc. London, B, Biol. Sci. 359: 183–195.

Hiraoka, K. & N. Tomaru. 2009. Population Genetic Structure of *Fagus* japonica Revealed by Nuclear Microsatellite Markers. Int. J. Plant Sci. 170: 748–758.

Iwasaki, T., K. Aoki, A. Seo & N. Murakami. 2006. Intraspecific sequence variation of chloroplast DNA among the component species of deciduous broad-leaved forests in Japan. J. Plant Res. 119: 539–552.

Kamei, T. & Research Group for the Biogeography from Würm Glacial. 1981. Fauna and flora of the Japanese islands in the last glacial time. Quaternary Res. 20: 191–205. (in Japanese)

McCauley, D. E. 1995. The use of chloroplast DNA polymorphism in studies of gene flow in plants. Trends Ecol. Evol. 10: 198–202.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Newton, A. C., T. R. Allnutt, A. C. M. Gillies, A. J. Lowe & R. A. Ennos. 1999. Molecular phylogeography, intraspecific variation and the conservation of tree species. Trends Ecol. Evol. 14: 140–145.

Nishizawa, T., Y. Watano. 2000. Primer pairs suitable for PCR-SSCP analysis of chloroplast DNA in angio-

sperms. J. Phytogeogr. Taxon. 48: 63–66.
Nozaki, R. & K. Okutomi. 1990. Geographical distribution and zonal interpretation of intermediate-temperate forests in eastern Japan. Jap. J. Ecol. 40: 57–69. (in Japanese)
Ohba, H. 2006. Carpinus. *In*: Iwatsuki K., D. E. Boufford & H. Ohba (eds.), Flora of Japan, vol. 2a, pp. 39–41. Kodansha, Tokyo, Japan.
Ohi, T., M. Wakabayashi, S. Wu & J. Murata. 2003. Phylogeography of *Stachyurus praecox* (Stachyuraceae) in the Japanese Archipelago Based on Chloroplast DNA Haplotypes. J. Jap. Bot. 78: 1–14.
Ohta, Y. & N. Yonekura. 1987. Coastal line. *In*: Japan Association for Quaternary Research (ed.), Quaternary Maps of Japan, pp. 70–72. University of Tokyo Press, Tokyo, Japan. (in Japanese)
Okaura, T. & K. Harada. 2002. Phylogeographical structure revealed by chloroplast DNA variation in Japanese Beech (*Fagus crenata* Blume). Heredity 88: 322–329.
Okaura, T., N. D. Quang, M. Ubukata & K. Harada. 2007. Phylogeographic structure and late Quaternary population history of the Japanese oak *Quercus mongolica* var. *crispula* and related species revealed by chloroplast DNA variation. Genes Genet. Syst. 82: 465–477.
Rieseberg, L. H. & D. E. Soltis. 1991. Phylogenetic consequences of cytoplasmic gene flow in Plants. Evol. Trends. Plants. 5: 65–84
Setoguchi, H. & H. Ohba. 1995. Phylogenetic relationships in *Crossostylis* (Rhizophoraceae) inferred from restriction site variation of chloroplast DNA. J. Plant Res. 108: 87–92.
Soltis, D. E., A. B. Morris, J. S. McLachlan, P. S. Manos & P. S. Soltis. 2006. Comparative phylogeography of unglaciated eastern North America. Mol. Ecol. 15: 4261–4293.
Taberlet, P., L. Fumagalli, A. G. Wust-Saucy & J. F. Cosson. 1998. Comparative phylogeography and postglacial colonization routes in Europe. Mol. Ecol. 7: 453–464.
Taberlet, P., L. Gielly, G. Pautou & J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol. Biol. 17: 1105–1109.
Takahara, H., S. Sugita, S. P. Harrison, N. Miyoshi, Y. Morita & T. Uchiyama. 2000. Pollen-based reconstructions of Japanese biomes at 0,6000 and 18,000 ^{14}C yr BP. J. Biogeogr. 27: 665–683.
Tsukada, M. 1988. Japan. *In*: Huntley, B. & T. Webb III (eds.), Vegetation history, pp. 459–518. Kluwer Academic Publishers, London.
Yasuda, Y. & N. Miyoshi. 1998. Vegetation history in the Japanese Archipelago. Asakura, Tokyo. (in Japanese)

Received November 12, 2009; Accepted May 6, 2010

Appendix 1. Geographical information voucher information regarding the plant samples.

Species and site number	Locality	Coordinates	Altitude (m)	Haplotype	Voucher specimen number
Carpinus japonica Blume					
1	Tohoku, Miyagi Pref., Sendai, Sakunami	38°19′N, 140°38′E	300	A′	10769*
	Tohoku, Miyagi Pref., Sendai, Sakunami	38°19′N, 140°38′E	300	A′	10769*
	Tohoku, Miyagi Pref., Sendai, Sakunami	38°19′N, 140°38′E	300	A′	10769*
	Tohoku, Miyagi Pref., Sendai, Sakunami	38°19′N, 140°38′E	300	A′	10769*
2	Tohoku, Miyagi Pref., Marumori, the Matsuzaka Pass	37°50′N, 140°41′E	500	A′	10788*
	Tohoku, Miyagi Pref., Marumori, the Matsuzaka Pass	37°50′N, 140°41′E	500	A′	10788*
	Tohoku, Miyagi Pref., Marumori, the Matsuzaka Pass	37°50′N, 140°41′E	500	A′	10788*
	Tohoku, Miyagi Pref., Marumori, the Matsuzaka Pass	37°50′N, 140°41′E	500	A′	10788*
	Tohoku, Miyagi Pref., Marumori, the Matsuzaka Pass	37°50′N, 140°41′E	500	A′	10788*
	Tohoku, Miyagi Pref., Marumori, the Matsuzaka Pass	37°50′N, 140°41′E	500	A′	10788*
	Tohoku, Miyagi Pref., Marumori, the Matsuzaka Pass	37°50′N, 140°41′E	500	A′	10788*

Species and site number	Locality	Coordinates	Altitude (m)	Haplotype	Voucher specimen number
3	Tohoku, Fukushima Pref., Ootama, Amagasawa	37°35′N, 140°19′E	750	A′	4982
	Tohoku, Fukushima Pref., Ootama, Amagasawa	37°35′N, 140°19′E	750	A′	4988
	Tohoku, Fukushima Pref., Ootama, Amagasawa	37°35′N, 140°19′E	750	A′	4997
	Tohoku, Fukushima Pref., Ootama, Amagasawa	37°35′N, 140°19′E	750	A′	5026
4	Kanto, Ibaraki Pref., Daigo, Mt. Yamizo	36°55′N, 140°16′E	900	A	4931
	Kanto, Ibaraki Pref., Daigo, Mt. Yamizo	36°55′N, 140°16′E	900	A	4938
	Kanto, Ibaraki Pref., Daigo, Mt. Yamizo	36°55′N, 140°16′E	900	A′	4912
	Kanto, Ibaraki Pref., Daigo, Mt. Yamizo	36°55′N, 140°16′E	900	A′	4947
5	Kanto, Ibaraki Pref., Tsukuba, Mt. Tsukuba	36°13′N, 140°06′E	800	A	4873
	Kanto, Ibaraki Pref., Tsukuba, Mt. Tsukuba	36°13′N, 140°06′E	800	A	4895
	Kanto, Ibaraki Pref., Tsukuba, Mt. Tsukuba	36°13′N, 140°06′E	800	A′	4908
	Kanto, Ibaraki Pref., Tsukuba, Mt. Tsukuba	36°13′N, 140°06′E	800	C	4875#
	Kanto, Ibaraki Pref., Tsukuba, Mt. Tsukuba	36°13′N, 140°06′E	800	C	4897
6	Kanto, Tochigi Pref., Nikko, Mt. Nakimushi	36°44′N, 139°35′E	900	A′	2141
	Kanto, Tochigi Pref., Nikko, Mt. Nakimushi	36°44′N, 139°35′E	900	A′	2169
	Kanto, Tochigi Pref., Nikko, Mt. Nakimushi	36°44′N, 139°35′E	900	A′	2231
	Kanto, Tochigi Pref., Nikko, Mt. Nakimushi	36°44′N, 139°35′E	900	A′	2232
	Kanto, Tochigi Pref., Nikko, Mt. Nakimushi	36°44′N, 139°35′E	900	A′	2341
	Kanto, Tochigi Pref., Nikko, Mt. Nakimushi	36°44′N, 139°35′E	900	A′	2356
	Kanto, Tochigi Pref., Nikko, Mt. Nakimushi	36°44′N, 139°35′E	900	A′	2395
	Kanto, Tochigi Pref., Nikko, Mt. Nakimushi	36°44′N, 139°35′E	900	A′	2398
	Kanto, Tochigi Pref., Nikko, Mt. Nakimushi	36°44′N, 139°35′E	900	A′	2405
7	Kanto, Tochigi Pref., Nikko, Utagahama	36°44′N, 139°29′E	1300	A′	8749
8	Kanto, Gunma Pref., Niiharu, The Akatanigoe Pass	36°45′N, 138°54′E	850	A′	8848
	Kanto, Gunma Pref., Niiharu, The Akatanigoe Pass	36°45′N, 138°54′E	850	A′	8904
	Kanto, Gunma Pref., Niiharu, The Akatanigoe Pass	36°45′N, 138°54′E	850	A′	8910
	Kanto, Gunma Pref., Niiharu, The Akatanigoe Pass	36°45′N, 138°54′E	850	A′	8921
	Kanto, Gunma Pref., Niiharu, The Akatanigoe Pass	36°45′N, 138°54′E	850	A′	8779
	Kanto, Gunma Pref., Niiharu, The Akatanigoe Pass	36°45′N, 138°54′E	850	A′	8781
	Kanto, Gunma Pref., Niiharu, The Akatanigoe Pass	36°45′N, 138°54′E	850	A′	8782
9	Kanto, Gunma Pref., Akagi, Mt. Akagi	36°31′N, 139°08′E	1000	A′	4802
	Kanto, Gunma Pref., Akagi, Mt. Akagi	36°31′N, 139°08′E	1000	A′	4832
	Kanto, Gunma Pref., Akagi, Mt. Akagi	36°31′N, 139°08′E	1000	A′	4837
	Kanto, Gunma Pref., Akagi, Mt. Akagi	36°31′N, 139°08′E	1000	A′	4838
	Kanto, Gunma Pref., Akagi, Mt. Akagi	36°31′N, 139°08′E	1000	A′	4842
10	Kanto, Tokyo Pref., Hinohara, The Sayaguchi Pass	35°44′N, 139°01′E	1200	A′	4756
	Kanto, Tokyo Pref., Hinohara, The Sayaguchi Pass	35°44′N, 139°01′E	1200	A′	4777
	Kanto, Tokyo Pref., Hinohara, The Sayaguchi Pass	35°44′N, 139°01′E	1200	A′	4782
	Kanto, Tokyo Pref., Hinohara, The Sayaguchi Pass	35°44′N, 139°01′E	1200	A′	4760
	Kanto, Tokyo Pref., Hinohara, The Sayaguchi Pass	35°44′N, 139°01′E	1200	A′	4790
11	Hokuriku, Niigata Pref., Yuzawa, Tsuchitaru	36°52′N, 138°52′E	600	A′	9033
	Hokuriku, Niigata Pref., Yuzawa, Tsuchitaru	36°52′N, 138°52′E	600	A′	9036
12	Hokuriku, Fukui Pref., Takahama, Mt. Aoba	35°30′N, 135°29′E	650	B	4021
	Hokuriku, Fukui Pref., Takahama, Mt. Aoba	35°30′N, 135°29′E	650	B	4020
	Hokuriku, Fukui Pref., Takahama, Mt. Aoba	35°30′N, 135°29′E	650	D	4023#
13	Chubu, Nagano Pref., Otari, Kamaike	36°52′N, 137°58′E	1150	A	5652
14	Chubu, Nagano Pref., Otari, Kitano	36°49′N, 137°53′E	700	A	9150
	Chubu, Nagano Pref., Otari, Kitano	36°49′N, 137°53′E	700	A	9156
	Chubu, Nagano Pref., Otari, Kitano	36°49′N, 137°53′E	700	A	9164
	Chubu, Nagano Pref., Otari, Kitano	36°49′N, 137°53′E	700	A	9166

Species and site number	Locality	Coordinates	Altitude (m)	Haplotype	Voucher specimen number
	Chubu, Nagano Pref., Otari, Kitano	36°49′N, 137°53′E	700	A	9206
15	Chubu, Nagano Pref., Koumi, Inako	36°02′N, 138°26′E	1150	A	4584
	Chubu, Nagano Pref., Koumi, Inako	36°02′N, 138°26′E	1150	A	4596
	Chubu, Nagano Pref., Koumi, Inako	36°02′N, 138°26′E	1150	A	4602
	Chubu, Nagano Pref., Koumi, Inako	36°02′N, 138°26′E	1150	A	4607
	Chubu, Nagano Pref., Koumi, Inako	36°02′N, 138°26′E	1150	A	4593
16	Chubu, Nagano Pref., Omachi, Mt. Koguma	36°33′N, 137°49′E	1150	A	9073
	Chubu, Nagano Pref., Omachi, Mt. Koguma	36°33′N, 137°49′E	1150	A	9078
	Chubu, Nagano Pref., Omachi, Mt. Koguma	36°33′N, 137°49′E	1150	A	9080
	Chubu, Nagano Pref., Omachi, Mt. Koguma	36°33′N, 137°49′E	1150	A	9083
	Chubu, Nagano Pref., Omachi, Mt. Koguma	36°33′N, 137°49′E	1150	A	9094
17	Chubu, Yamanashi Pref., Hokuto, Kiyosato	35°53′N, 138°26′E	1100	A	4632
18	Chubu, Yamanashi Pref., Kamikuisiki, Motosu, Mt. Ryugatake	35°27′N, 138°35′E	1100	A	4650
	Chubu, Yamanashi Pref., Kamikuisiki, Motosu, Mt. Ryugatake	35°27′N, 138°35′E	1100	A′	4698
	Chubu, Yamanashi Pref., Kamikuisiki, Motosu, Mt. Ryugatake	35°27′N, 138°35′E	1100	A′	4648
19	Chubu, Shizuoka Pref., Kawadu, Mt. Amagi	34°50′N, 138°57′E	1150	A	4706#
	Chubu, Shizuoka Pref., Kawadu, Mt. Amagi	34°50′N, 138°57′E	1150	A	4726
	Chubu, Shizuoka Pref., Kawadu, Mt. Amagi	34°50′N, 138°57′E	1150	A	4729
	Chubu, Shizuoka Pref., Kawadu, Mt. Amagi	34°50′N, 138°57′E	1150	A′	4737
20	Chubu, Nagano Pref., Ohtaki, Hyougase	35°48′N, 137°30′E	1200	A	4542
	Chubu, Nagano Pref., Ohtaki, Hyougase	35°48′N, 137°30′E	1200	A	4544
	Chubu, Nagano Pref., Ohtaki, Hyougase	35°48′N, 137°30′E	1200	A	4555
21	Chubu, Gifu Pref., Shirakawa, Shiramizunotaki	36°08′N, 136°49′E	1150	A	5603
	Chubu, Gifu Pref., Shirakawa, Shiramizunotaki	36°08′N, 136°49′E	1150	A	5610
	Chubu, Gifu Pref., Shirakawa, Shiramizunotaki	36°08′N, 136°49′E	1150	A	5615
	Chubu, Gifu Pref., Shirakawa, Shiramizunotaki	36°08′N, 136°49′E	1150	A	5625
	Chubu, Gifu Pref., Shirakawa, Shiramizunotaki	36°08′N, 136°49′E	1150	A	5629
22	Chubu, Nagano Pref., Urugi, Mt. Chausu	35°13′N, 137°39′E	1250	A	4471
	Chubu, Nagano Pref., Urugi, Mt. Chausu	35°13′N, 137°39′E	1250	A	4491
	Chubu, Nagano Pref., Urugi, Mt. Chausu	35°13′N, 137°39′E	1250	A	4497
	Chubu, Nagano Pref., Urugi, Mt. Chausu	35°13′N, 137°39′E	1250	A	4512
	Chubu, Nagano Pref., Urugi, Mt. Chausu	35°13′N, 137°39′E	1250	A	4519
	Chubu, Nagano Pref., Urugi, Mt. Chausu	35°13′N, 137°39′E	1250	A	4520
23	Chubu, Gifu Pref., Hachiman, Miyama	35°44′N, 136°59′E	600	A	5578
	Chubu, Gifu Pref., Hachiman, Miyama	35°44′N, 136°59′E	600	A	5586
24	Kinki, Shiga Pref., Yogo, Mt. Yokoyamadake	35°36′N, 136°15′E	1050	A	4068
	Kinki, Shiga Pref., Yogo, Mt. Yokoyamadake	35°36′N, 136°15′E	1050	A	4069
	Kinki, Shiga Pref., Yogo, Mt. Yokoyamadake	35°36′N, 136°15′E	1050	A	4070
	Kinki, Shiga Pref., Yogo, Mt. Yokoyamadake	35°36′N, 136°15′E	1050	A	4071
	Kinki, Shiga Pref., Yogo, Mt. Yokoyamadake	35°36′N, 136°15′E	1050	A	4072
	Kinki, Shiga Pref., Yogo, Mt. Yokoyamadake	35°36′N, 136°15′E	1050	A	4074
	Kinki, Shiga Pref., Yogo, Mt. Yokoyamadake	35°36′N, 136°15′E	1050	A	4076
	Kinki, Shiga Pref., Yogo, Mt. Yokoyamadake	35°36′N, 136°15′E	1050	A	4077
	Kinki, Shiga Pref., Yogo, Mt. Yokoyamadake	35°36′N, 136°15′E	1050	A	4173
	Kinki, Shiga Pref., Yogo, Mt. Yokoyamadake	35°36′N, 136°15′E	1050	A	4174
	Kinki, Shiga Pref., Yogo, Mt. Yokoyamadake	35°36′N, 136°15′E	1050	A	4175
	Kinki, Shiga Pref., Yogo, Mt. Yokoyamadake	35°36′N, 136°15′E	1050	A	4176

Species and site number	Locality	Coordinates	Altitude (m)	Haplotype	Voucher specimen number
	Kinki, Shiga Pref., Yogo, Mt. Yokoyamadake	35°36′N, 136°15′E	1050	A	4177
	Kinki, Shiga Pref., Yogo, Mt. Yokoyamadake	35°36′N, 136°15′E	1050	A	4073
	Kinki, Shiga Pref., Yogo, Mt. Yokoyamadake	35°36′N, 136°15′E	1050	A	4075
25	Kinki, Shiga Pref., Yohkaichi, Mt. Watamuke	35°01′N, 136°20′E	1050	A	3682
	Kinki, Shiga Pref., Yohkaichi, Mt. Watamuke	35°01′N, 136°20′E	1050	A	3730
	Kinki, Shiga Pref., Yohkaichi, Mt. Watamuke	35°01′N, 136°20′E	1050	A	3731
	Kinki, Shiga Pref., Yohkaichi, Mt. Watamuke	35°01′N, 136°20′E	1050	A	3741
	Kinki, Shiga Pref., Yohkaichi, Mt. Watamuke	35°01′N, 136°20′E	1050	A	3755
	Kinki, Shiga Pref., Yohkaichi, Mt. Watamuke	35°01′N, 136°20′E	1050	A	3760
	Kinki, Shiga Pref., Yohkaichi, Mt. Watamuke	35°01′N, 136°20′E	1050	A	3771
	Kinki, Shiga Pref., Yohkaichi, Mt. Watamuke	35°01′N, 136°20′E	1050	A	3726
26	Kinki, Kyoto Pref., Yasaka, Mt. Taiko	35°41′N, 135°12′E	650	A	3950
	Kinki, Kyoto Pref., Yasaka, Mt. Taiko	35°41′N, 135°12′E	650	B	3951
27	Kinki, Kyoto Pref., Kita-ku, Kumogahata, the Mochikoshi Pass	35°06′N, 135°42′E	400	A	4230
	Kinki, Kyoto Pref., Kita-ku, Kumogahata, the Mochikoshi Pass	35°06′N, 135°42′E	400	A	4231
28	Kinki, Kyoto Pref., Ukyo-ku, Umegahata	35°03′N, 136°41′E	400	A	4227
29	Kinki, Kyoto Pref., Fukuchiyama, Mt. Ooe	35°27′N, 135°06′E	800	A	3868
	Kinki, Kyoto Pref., Fukuchiyama, Mt. Ooe	35°27′N, 135°06′E	800	B	3834
	Kinki, Kyoto Pref., Fukuchiyama, Mt. Ooe	35°27′N, 135°06′E	800	B	3867
	Kinki, Kyoto Pref., Fukuchiyama, Mt. Ooe	35°27′N, 135°06′E	800	B	3869
	Kinki, Kyoto Pref., Fukuchiyama, Mt. Ooe	35°27′N, 135°06′E	800	B	3872
	Kinki, Kyoto Pref., Fukuchiyama, Mt. Ooe	35°27′N, 135°06′E	800	B	3874
	Kinki, Kyoto Pref., Fukuchiyama, Mt. Ooe	35°27′N, 135°06′E	800	B	3875
	Kinki, Kyoto Pref., Fukuchiyama, Mt. Ooe	35°27′N, 135°06′E	800	B	3871
30	Kinki, Nara Pref., Kawakami, Shionoha	34°13′N, 136°05′E	500	A	8476
	Kinki, Nara Pref., Kawakami, Shionoha	34°13′N, 136°05′E	500	A	8477
	Kinki, Nara Pref., Kawakami, Shionoha	34°13′N, 136°05′E	500	A	8478
	Kinki, Nara Pref., Kawakami, Shionoha	34°13′N, 136°05′E	500	A	8479
	Kinki, Nara Pref., Kawakami, Shionoha	34°13′N, 136°05′E	500	A	8482
31	Kinki, Nara Pref., Kamikitayama, Mt. Wasamata	34°13′N, 135°59′E	1000	A	5520
32	Kinki, Nara Pref., Gose, Mt. Kongo	34°25′N, 135°40′E	1000	A	5504
	Kinki, Nara Pref., Gose, Mt. Kongo	34°25′N, 135°40′E	1000	A	5510
33	Kinki, Hyogo Pref., Sekinomiya, Mt. Hachibuse	35°23′N, 134°32′E	1000	B	5739
34	Kinki, Hyogo Pref., Ichinomiya, Mt. Fujinashi	35°16′N, 134°35′E	900	B	3541
	Kinki, Hyogo Pref., Ichinomiya, Mt. Fujinashi	35°16′N, 134°35′E	900	B	3543
	Kinki, Hyogo Pref., Ichinomiya, Mt. Fujinashi	35°16′N, 134°35′E	900	B	3546
	Kinki, Hyogo Pref., Ichinomiya, Mt. Fujinashi	35°16′N, 134°35′E	900	B	3640
	Kinki, Hyogo Pref., Ichinomiya, Mt. Fujinashi	35°16′N, 134°35′E	900	B	3642
	Kinki, Hyogo Pref., Ichinomiya, Mt. Fujinashi	35°16′N, 134°35′E	900	B	3659
	Kinki, Hyogo Pref., Ichinomiya, Mt. Fujinashi	35°16′N, 134°35′E	900	B	3542
	Kinki, Hyogo Pref., Ichinomiya, Mt. Fujinashi	35°16′N, 134°35′E	900	B	3556
	Kinki, Hyogo Pref., Ichinomiya, Mt. Fujinashi	35°16′N, 134°35′E	900	B	3615
	Kinki, Hyogo Pref., Ichinomiya, Mt. Fujinashi	35°16′N, 134°35′E	900	B	3620
35	Kinki, Nara Pref., Totsukawa, Mt. Obakodake	34°04′N, 135°38′E	1250	A	8558
	Kinki, Nara Pref., Totsukawa, Mt. Obakodake	34°04′N, 135°38′E	1250	A	8563
	Kinki, Nara Pref., Totsukawa, Mt. Obakodake	34°04′N, 135°38′E	1250	A′	8559
	Kinki, Nara Pref., Totsukawa, Mt. Obakodake	34°04′N, 135°38′E	1250	A′	8565
36	Chugoku, Okayama Pref., Shinjyo, Nodorodawa	35°14′N, 133°34′E	900	B	5753

Species and site number	Locality	Coordinates	Altitude (m)	Haplotype	Voucher specimen number
	Chugoku, Okayama Pref., Shinjyo, Nodorodawa	35°14′N, 133°34′E	900	B	5755
37	Chugoku, Okayama Pref., Shinjyo, Mt. Kenashi	35°14′N, 133°30′E	1200	B	7782
	Chugoku, Okayama Pref., Shinjyo, Mt. Kenashi	35°14′N, 133°30′E	1200	B	7802
	Chugoku, Okayama Pref., Shinjyo, Mt. Kenashi	35°14′N, 133°30′E	1200	B	7844
	Chugoku, Okayama Pref., Shinjyo, Mt. Kenashi	35°14′N, 133°30′E	1200	B	7865
	Chugoku, Okayama Pref., Shinjyo, Mt. Kenashi	35°14′N, 133°30′E	1200	B	7909
38	Chugoku, Hiroshima Pref., Toyohira, Mt. Ryuuzu	34°39′N, 132°25′E	900	B	5784
	Chugoku, Hiroshima Pref., Toyohira, Mt. Ryuuzu	34°39′N, 132°25′E	900	B	5788
39	Chugoku, Hiroshima Pref., Geihoku, Mt. Garyuu	34°41′N, 132°11′E	1050	B	5799
	Chugoku, Hiroshima Pref., Geihoku, Mt. Garyuu	34°41′N, 132°11′E	1050	B	5821
	Chugoku, Hiroshima Pref., Geihoku, Mt. Garyuu	34°41′N, 132°11′E	1050	B	5825
	Chugoku, Hiroshima Pref., Geihoku, Mt. Garyuu	34°41′N, 132°11′E	1050	B	5839
	Chugoku, Hiroshima Pref., Geihoku, Mt. Garyuu	34°41′N, 132°11′E	1050	B	5846
40	Chugoku, Yamaguchi Pref., Kano, Mt. Nagano	34°16′N, 131°52′E	1000	B	8191
	Chugoku, Yamaguchi Pref., Kano, Mt. Nagano	34°16′N, 131°52′E	1000	B	8225
41	Shikoku, Kagawa Pref., Shionoe, Mt. Ohtaki	34°07′N, 134°07′E	950	A′	4258
	Shikoku, Kagawa Pref., Shionoe, Mt. Ohtaki	34°07′N, 134°07′E	950	A′	4259
	Shikoku, Kagawa Pref., Shionoe, Mt. Ohtaki	34°07′N, 134°07′E	950	A′	4261
	Shikoku, Kagawa Pref., Shionoe, Mt. Ohtaki	34°07′N, 134°07′E	950	A′	4263
	Shikoku, Kagawa Pref., Shionoe, Mt. Ohtaki	34°07′N, 134°07′E	950	A′	4264
	Shikoku, Kagawa Pref., Shionoe, Mt. Ohtaki	34°07′N, 134°07′E	950	B	4262
42	Shikoku, Kochi Pref., Monobe, Mt. Shiraga	33°48′N, 133°58′E	1000	A′	4424
	Shikoku, Kochi Pref., Monobe, Mt. Shiraga	33°48′N, 133°58′E	1000	A′	4425
	Shikoku, Kochi Pref., Monobe, Mt. Shiraga	33°48′N, 133°58′E	1000	A′	4431
	Shikoku, Kochi Pref., Monobe, Mt. Shiraga	33°48′N, 133°58′E	1000	B	4428#
43	Shikoku, Kochi Pref., Hongawa, Mt. Kanpuu	33°48′N, 133°15′E	1400	B	4312
	Shikoku, Kochi Pref., Hongawa, Mt. Kanpuu	33°48′N, 133°15′E	1400	B	4313
	Shikoku, Kochi Pref., Hongawa, Mt. Kanpuu	33°48′N, 133°15′E	1400	B	4314
	Shikoku, Kochi Pref., Hongawa, Mt. Kanpuu	33°48′N, 133°15′E	1400	B	4315
	Shikoku, Kochi Pref., Hongawa, Mt. Kanpuu	33°48′N, 133°15′E	1400	B	4316
	Shikoku, Kochi Pref., Hongawa, Mt. Kanpuu	33°48′N, 133°15′E	1400	B	4317
	Shikoku, Kochi Pref., Hongawa, Mt. Kanpuu	33°48′N, 133°15′E	1400	B	4318
44	Shikoku, Ehime Pref., Omogo, Koami	33°44′N, 133°00′E	750	B	4356
45	Shikoku, Ehime Pref., Shigenobu, Mt. Saragamine	33°43′N, 132°53′E	1100	B	4377
	Shikoku, Ehime Pref., Shigenobu, Mt. Saragamine	33°43′N, 132°53′E	1100	B	4379
	Shikoku, Ehime Pref., Shigenobu, Mt. Saragamine	33°43′N, 132°53′E	1100	B	4380
	Shikoku, Ehime Pref., Shigenobu, Mt. Saragamine	33°43′N, 132°53′E	1100	B	4381
	Shikoku, Ehime Pref., Shigenobu, Mt. Saragamine	33°43′N, 132°53′E	1100	B	4382
46	Shikoku, Kochi Pref., Higashitsuno, Mt. Irazu	33°26′N, 133°03′E	1300	B	373
	Shikoku, Kochi Pref., Higashitsuno, Mt. Irazu	33°26′N, 133°03′E	1300	B	391
	Shikoku, Kochi Pref., Higashitsuno, Mt. Irazu	33°26′N, 133°03′E	1300	B	458
	Shikoku, Kochi Pref., Higashitsuno, Mt. Irazu	33°26′N, 133°03′E	1300	B	487
	Shikoku, Kochi Pref., Higashitsuno, Mt. Irazu	33°26′N, 133°03′E	1300	B	516
	Shikoku, Kochi Pref., Higashitsuno, Mt. Irazu	33°26′N, 133°03′E	1300	B	519
	Shikoku, Kochi Pref., Higashitsuno, Mt. Irazu	33°26′N, 133°03′E	1300	B	535
	Shikoku, Kochi Pref., Higashitsuno, Mt. Irazu	33°26′N, 133°03′E	1300	B	536
	Shikoku, Kochi Pref., Higashitsuno, Mt. Irazu	33°26′N, 133°03′E	1300	B	537
47	Kyushu, Oita Pref., Takeda, Bogaturu	33°07′N, 131°15′E	1100	B	6288
	Kyushu, Oita Pref., Takeda, Bogaturu	33°07′N, 131°15′E	1100	B	6347
	Kyushu, Oita Pref., Takeda, Bogaturu	33°07′N, 131°15′E	1100	B	6353

Species and site number	Locality	Coordinates	Altitude (m)	Haplotype	Voucher specimen number
	Kyushu, Oita Pref., Takeda, Bogaturu	33°07′N, 131°15′E	1100	B	6434
48	Kyushu, Fukuoka Pref., Soeda, Mt. Hiko	33°28′N, 130°55′E	1000	B	5897
	Kyushu, Fukuoka Pref., Soeda, Mt. Hiko	33°28′N, 130°55′E	1000	B	5904
49	Kyushu, Oita Pref., Takeda, Mt. Sobo	32°50′N, 131°20′E	900	B	7591
	Kyushu, Oita Pref., Takeda, Mt. Sobo	32°50′N, 131°20′E	900	B	7623
50	Kyushu, Fukuoka Pref., Hukuoka, Mt. Sehuri	33°26′N, 130°22′E	1000	B	5853
	Kyushu, Fukuoka Pref., Hukuoka, Mt. Sehuri	33°26′N, 130°22′E	1000	B	5866
51	Kyushu, Miyazaki Pref., Shiiba, The Ookouchi Pass	32°23′N, 131°11′E	1100	B	7522
	Kyushu, Miyazaki Pref., Shiiba, The Ookouchi Pass	32°23′N, 131°11′E	1100	B	7541
52	Kyushu, Kagoshima Pref., Miyanojyo, Mt. Shibi	31°58′N, 130°22′E	1050	B	7209
	Kyushu, Kagoshima Pref., Miyanojyo, Mt. Shibi	31°58′N, 130°22′E	1050	B	7207
Carpinus tschonoskii Maxim.					
1	Tohoku, Iwate Pref., Murone, Mt. Murone	38°58′N, 141°27′E	600	D	5123
	Tohoku, Iwate Pref., Murone, Mt. Murone	38°58′N, 141°27′E	600	D	5125
2	Tohoku, Miyagi Pref., Kitakami, Mt. Okinakura	38°36′N, 141°23′E	500	D	5097#
	Tohoku, Miyagi Pref., Kitakami, Mt. Okinakura	38°36′N, 141°23′E	500	D	5103
3	Tohoku, Miyagi Pref., Kurikoma, Yanagisawa	38°54′N, 140°51′E	450	D	5143
	Tohoku, Miyagi Pref., Kurikoma, Yanagisawa	38°54′N, 140°51′E	450	D	5144
4	Tohoku, Miyagi Pref., Marumori, the Matsuzaka Pass	37°50′N, 140°41′E	500	A	10790*
	Tohoku, Miyagi Pref., Marumori, the Matsuzaka Pass	37°50′N, 140°41′E	500	A	10790*
	Tohoku, Miyagi Pref., Marumori, the Matsuzaka Pass	37°50′N, 140°41′E	500	A	10790*
	Tohoku, Miyagi Pref., Marumori, the Matsuzaka Pass	37°50′N, 140°41′E	500	A	10790*
	Tohoku, Miyagi Pref., Marumori, the Matsuzaka Pass	37°50′N, 140°41′E	500	A	10790*
5	Kanto, Ibaraki Pref., Daigo, Mt. Yamizo	36°55′N, 140°16′E	900	A	4911
	Kanto, Ibaraki Pref., Daigo, Mt. Yamizo	36°55′N, 140°16′E	900	A	4923
	Kanto, Ibaraki Pref., Daigo, Mt. Yamizo	36°55′N, 140°16′E	900	A	4925
	Kanto, Ibaraki Pref., Daigo, Mt. Yamizo	36°55′N, 140°16′E	900	A	4929
	Kanto, Ibaraki Pref., Daigo, Mt. Yamizo	36°55′N, 140°16′E	900	A	4933
	Kanto, Ibaraki Pref., Daigo, Mt. Yamizo	36°55′N, 140°16′E	900	A	4939
	Kanto, Ibaraki Pref., Daigo, Mt. Yamizo	36°55′N, 140°16′E	900	A	4953
6	Kanto, Tochigi Pref., Kuriyama, Mt. Katsurou	36°55′N, 139°40′E	1100	A	9554*
	Kanto, Tochigi Pref., Kuriyama, Mt. Katsurou	36°55′N, 139°40′E	1100	A	9554*
	Kanto, Tochigi Pref., Kuriyama, Mt. Katsurou	36°55′N, 139°40′E	1100	A	9554*
	Kanto, Tochigi Pref., Kuriyama, Mt. Katsurou	36°55′N, 139°40′E	1100	A	9554*
7	Kanto, Ibaraki Pref., Tsukuba, Mt. Tsukuba	36°13′N, 140°06′E	800	A	4874
	Kanto, Ibaraki Pref., Tsukuba, Mt. Tsukuba	36°13′N, 140°06′E	800	A	4877
	Kanto, Ibaraki Pref., Tsukuba, Mt. Tsukuba	36°13′N, 140°06′E	800	A	4882
	Kanto, Ibaraki Pref., Tsukuba, Mt. Tsukuba	36°13′N, 140°06′E	800	A	4888
	Kanto, Ibaraki Pref., Tsukuba, Mt. Tsukuba	36°13′N, 140°06′E	800	A	4894
	Kanto, Ibaraki Pref., Tsukuba, Mt. Tsukuba	36°13′N, 140°06′E	800	A	4898
8	Kanto, Gunma Pref., Akagi, Mt. Akagi	36°31′N, 139°08′E	1000	A	4805
	Kanto, Gunma Pref., Akagi, Mt. Akagi	36°31′N, 139°08′E	1000	A	4816
	Kanto, Gunma Pref., Akagi, Mt. Akagi	36°31′N, 139°08′E	1000	A	4831
9	Kanto, Tokyo Pref., Hinohara, The Sayaguchi Pass	35°44′N, 139°01′E	1200	A	4744
	Kanto, Tokyo Pref., Hinohara, The Sayaguchi Pass	35°44′N, 139°01′E	1200	A	4751
	Kanto, Tokyo Pref., Hinohara, The Sayaguchi Pass	35°44′N, 139°01′E	1200	A″	4789
10	Hokuriku, Toyama Pref., Namerikawa, Tofukiji	36°43′N, 137°24′E	200	A′	10460*
	Hokuriku, Toyama Pref., Namerikawa, Tofukiji	36°43′N, 137°24′E	200	A′	10460*
	Hokuriku, Toyama Pref., Namerikawa, Tofukiji	36°43′N, 137°24′E	200	A′	10460*

Species and site number	Locality	Coordinates	Altitude (m)	Haplotype	Voucher specimen number
	Hokuriku, Toyama Pref., Namerikawa, Tofukiji	36°43′N, 137°24′E	200	A′	10460*
11	Hokuriku, Fukui Pref., Takahama, Mt. Aoba	35°30′N, 135°29′E	650	A′	4011
	Hokuriku, Fukui Pref., Takahama, Mt. Aoba	35°30′N, 135°29′E	650	A′	4012
	Hokuriku, Fukui Pref., Takahama, Mt. Aoba	35°30′N, 135°29′E	650	A′	4014
	Hokuriku, Fukui Pref., Takahama, Mt. Aoba	35°30′N, 135°29′E	650	B	4010
	Hokuriku, Fukui Pref., Takahama, Mt. Aoba	35°30′N, 135°29′E	650	B	4013
12	Chubu, Yamanashi Pref., Hokuto, along the Shiozawa River	35°51′N, 138°15′E	1100	A	10034
	Chubu, Yamanashi Pref., Hokuto, along the Shiozawa River	35°51′N, 138°15′E	1100	A	10036
	Chubu, Yamanashi Pref., Hokuto, along the Shiozawa River	35°51′N, 138°15′E	1100	A	10030
	Chubu, Yamanashi Pref., Hokuto, along the Shiozawa River	35°51′N, 138°15′E	1100	A	10035
13	Chubu, Yamanashi Pref., Kamikuisiki, Motosu, Mt. Ryugatake	35°27′N, 138°35′E	1100	A	4652
	Chubu, Yamanashi Pref., Kamikuisiki, Motosu, Mt. Ryugatake	35°27′N, 138°35′E	1100	A	4658
	Chubu, Yamanashi Pref., Kamikuisiki, Motosu, Mt. Ryugatake	35°27′N, 138°35′E	1100	A	4659
	Chubu, Yamanashi Pref., Kamikuisiki, Motosu, Mt. Ryugatake	35°27′N, 138°35′E	1100	A	4663
	Chubu, Yamanashi Pref., Kamikuisiki, Motosu, Mt. Ryugatake	35°27′N, 138°35′E	1100	A	4668
14	Chubu, Shizuoka Pref., Kawadu, Mt. Amagi	34°50′N, 138°57′E	1150	A	4711#
	Chubu, Shizuoka Pref., Kawadu, Mt. Amagi	34°50′N, 138°57′E	1150	A	4720
	Chubu, Shizuoka Pref., Kawadu, Mt. Amagi	34°50′N, 138°57′E	1150	A	4725
	Chubu, Shizuoka Pref., Kawadu, Mt. Amagi	34°50′N, 138°57′E	1150	A	4738
15	Chubu, Nagano Pref., Kiso, the Torii Pass	35°57′N, 137°47′E	1200	A	9858
	Chubu, Nagano Pref., Kiso, the Torii Pass	35°57′N, 137°47′E	1200	A	9859
16	Chubu, Nagano Pref., Ohtaki, Hyougase	35°48′N, 137°30′E	1200	A	4532
	Chubu, Nagano Pref., Ohtaki, Hyougase	35°48′N, 137°30′E	1200	A	4564
17	Chubu, Gifu Pref., Nakatsugawa, Mt. Tengumori	35°24′N, 137°31′E	1200	A	9719*
	Chubu, Gifu Pref., Nakatsugawa, Mt. Tengumori	35°24′N, 137°31′E	1200	A	9719*
	Chubu, Gifu Pref., Nakatsugawa, Mt. Tengumori	35°24′N, 137°31′E	1200	A	9719*
18	Chubu, Nagano Pref., Urugi, Mt. Chausu	35°13′N, 137°39′E	1250	A	4451
	Chubu, Nagano Pref., Urugi, Mt. Chausu	35°13′N, 137°39′E	1250	A	4483
	Chubu, Nagano Pref., Urugi, Mt. Chausu	35°13′N, 137°39′E	1250	A	4494
	Chubu, Nagano Pref., Urugi, Mt. Chausu	35°13′N, 137°39′E	1250	A	4502
	Chubu, Nagano Pref., Urugi, Mt. Chausu	35°13′N, 137°39′E	1250	A	4511
19	Chubu, Gifu Pref., Seki, along the Itadori River	35°44′N, 136°45′E	400	A	10358*
	Chubu, Gifu Pref., Seki, along the Itadori River	35°44′N, 136°45′E	400	A′	10358*
	Chubu, Gifu Pref., Seki, along the Itadori River	35°44′N, 136°45′E	400	A′	10358*
20	Kinki, Shiga Pref., Yogo, Mt. Yokoyamadake	35°36′N, 136°15′E	1050	A	4067
	Kinki, Shiga Pref., Yogo, Mt. Yokoyamadake	35°36′N, 136°15′E	1050	A	4178
21	Kinki, Shiga Pref., Yohkaichi, Mt. Watamuke	35°01′N, 136°20′E	1050	A	3684
	Kinki, Shiga Pref., Yohkaichi, Mt. Watamuke	35°01′N, 136°20′E	1050	A	3752
	Kinki, Shiga Pref., Yohkaichi, Mt. Watamuke	35°01′N, 136°20′E	1050	A′	3683
	Kinki, Shiga Pref., Yohkaichi, Mt. Watamuke	35°01′N, 136°20′E	1050	A′	3717
	Kinki, Shiga Pref., Yohkaichi, Mt. Watamuke	35°01′N, 136°20′E	1050	A′	3740
	Kinki, Shiga Pref., Yohkaichi, Mt. Watamuke	35°01′N, 136°20′E	1050	A′	3746
	Kinki, Shiga Pref., Yohkaichi, Mt. Watamuke	35°01′N, 136°20′E	1050	A′	3747

Species and site number	Locality	Coordinates	Altitude (m)	Haplotype	Voucher specimen number
	Kinki, Shiga Pref., Yohkaichi, Mt. Watamuke	35°01′N, 136°20′E	1050	A′	3753
	Kinki, Shiga Pref., Yohkaichi, Mt. Watamuke	35°01′N, 136°20′E	1050	A′	3759
	Kinki, Shiga Pref., Yohkaichi, Mt. Watamuke	35°01′N, 136°20′E	1050	A′	3722
	Kinki, Shiga Pref., Yohkaichi, Mt. Watamuke	35°01′N, 136°20′E	1050	A′	3735
22	Kinki, Kyoto, Ine, Rokumanbu	35°42′N, 135°16′E	100	A′	3991
23	Kinki, Kyoto Pref., Yasaka, Mt. Taiko	35°41′N, 135°12′E	650	A′	3937
	Kinki, Kyoto Pref., Yasaka, Mt. Taiko	35°41′N, 135°12′E	650	A′	3938
	Kinki, Kyoto Pref., Yasaka, Mt. Taiko	35°41′N, 135°12′E	650	A′	3939
	Kinki, Kyoto Pref., Yasaka, Mt. Taiko	35°41′N, 135°12′E	650	A′	3940
	Kinki, Kyoto Pref., Yasaka, Mt. Taiko	35°41′N, 135°12′E	650	A′	3941
	Kinki, Kyoto Pref., Yasaka, Mt. Taiko	35°41′N, 135°12′E	650	A′	3942
	Kinki, Kyoto Pref., Yasaka, Mt. Taiko	35°41′N, 135°12′E	650	A′	3947
24	Kinki, Kyoto, Kyoto, Ukyo-ku, Umegahata	35°03′N, 136°41′E	400	A′	4226
25	Kinki, Kyoto Pref., Fukuchiyama, Mt. Ooe	35°27′N, 135°06′E	800	A′	3829
	Kinki, Kyoto Pref., Fukuchiyama, Mt. Ooe	35°27′N, 135°06′E	800	A′	3830
	Kinki, Kyoto Pref., Fukuchiyama, Mt. Ooe	35°27′N, 135°06′E	800	A′	3831
	Kinki, Kyoto Pref., Fukuchiyama, Mt. Ooe	35°27′N, 135°06′E	800	A′	3832
	Kinki, Kyoto Pref., Fukuchiyama, Mt. Ooe	35°27′N, 135°06′E	800	A′	3835
	Kinki, Kyoto Pref., Fukuchiyama, Mt. Ooe	35°27′N, 135°06′E	800	A′	3880
	Kinki, Kyoto Pref., Fukuchiyama, Mt. Ooe	35°27′N, 135°06′E	800	A′	3882
	Kinki, Kyoto Pref., Fukuchiyama, Mt. Ooe	35°27′N, 135°06′E	800	B	3881
26	Kinki, Nara Pref., Kawakami, Shionoha	34°13′N, 136°05′E	500	A′	8468
	Kinki, Nara Pref., Kawakami, Shionoha	34°13′N, 136°05′E	500	A′	8475
	Kinki, Nara Pref., Kawakami, Shionoha	34°13′N, 136°05′E	500	A′	8481
27	Kinki, Nara Pref., Kamikitayama, Mt. Wasamata	34°13′N, 135°59′E	1000	A′	5513
	Kinki, Nara Pref., Kamikitayama, Mt. Wasamata	34°13′N, 135°59′E	1000	A′	5514
	Kinki, Nara Pref., Kamikitayama, Mt. Wasamata	34°13′N, 135°59′E	1000	A′	5531
	Kinki, Nara Pref., Kamikitayama, Mt. Wasamata	34°13′N, 135°59′E	1000	A′	5534
28	Kinki, Nara Pref., Gose, Mt. Kongo	34°25′N, 135°40′E	1000	A′	5493
29	Kinki, Hyogo Pref., Sekinomiya, Mt. Hachibuse	35°23′N, 134°32′E	1000	B	5741
30	Kinki, Hyogo Pref., Ichinomiya, Mt. Fujinashi	35°16′N, 134°35′E	900	B	3545
	Kinki, Hyogo Pref., Ichinomiya, Mt. Fujinashi	35°16′N, 134°35′E	900	B	3559
	Kinki, Hyogo Pref., Ichinomiya, Mt. Fujinashi	35°16′N, 134°35′E	900	B	3616
	Kinki, Hyogo Pref., Ichinomiya, Mt. Fujinashi	35°16′N, 134°35′E	900	B	3617
	Kinki, Hyogo Pref., Ichinomiya, Mt. Fujinashi	35°16′N, 134°35′E	900	B	3632
	Kinki, Hyogo Pref., Ichinomiya, Mt. Fujinashi	35°16′N, 134°35′E	900	B	3633
	Kinki, Hyogo Pref., Ichinomiya, Mt. Fujinashi	35°16′N, 134°35′E	900	B	3641
	Kinki, Hyogo Pref., Ichinomiya, Mt. Fujinashi	35°16′N, 134°35′E	900	B	3643
	Kinki, Hyogo Pref., Ichinomiya, Mt. Fujinashi	35°16′N, 134°35′E	900	B	3515
	Kinki, Hyogo Pref., Ichinomiya, Mt. Fujinashi	35°16′N, 134°35′E	900	B	3530
31	Kinki, Nara Pref., Totsukawa, Mt. Obakodake	34°04′N, 135°38′E	1250	A	8544
	Kinki, Nara Pref., Totsukawa, Mt. Obakodake	34°04′N, 135°38′E	1250	A′	8531
	Kinki, Nara Pref., Totsukawa, Mt. Obakodake	34°04′N, 135°38′E	1250	A′	8540
	Kinki, Nara Pref., Totsukawa, Mt. Obakodake	34°04′N, 135°38′E	1250	A′	8548
32	Chugoku, Okayama Pref., Shinjyo, Nodorodawa	35°14′N, 133°34′E	900	B	5759
33	Chugoku, Okayama Pref., Shinjyo, Mt. Kenashi	35°14′N, 133°30′E	1200	B	7887
	Chugoku, Okayama Pref., Shinjyo, Mt. Kenashi	35°14′N, 133°30′E	1200	B	7910
	Chugoku, Okayama Pref., Shinjyo, Mt. Kenashi	35°14′N, 133°30′E	1200	B	7882
34	Chugoku, Hiroshima Pref., Toyohira, Mt. Ryuuzu	34°39′N, 132°25′E	900	B	5786
	Chugoku, Hiroshima Pref., Toyohira, Mt. Ryuuzu	34°39′N, 132°25′E	900	B	5789

Species and site number	Locality	Coordinates	Altitude (m)	Haplotype	Voucher specimen number
	Chugoku, Hiroshima Pref., Toyohira, Mt. Ryuuzu	34°39′N, 132°25′E	900	B	5792
	Chugoku, Hiroshima Pref., Toyohira, Mt. Ryuuzu	34°39′N, 132°25′E	900	B	5793
35	Chugoku, Hiroshima Pref., Geihoku, Mt. Garyuu	34°41′N, 132°11′E	1050	B	5818
	Chugoku, Hiroshima Pref., Geihoku, Mt. Garyuu	34°41′N, 132°11′E	1050	B	5830
	Chugoku, Hiroshima Pref., Geihoku, Mt. Garyuu	34°41′N, 132°11′E	1050	B	5835
	Chugoku, Hiroshima Pref., Geihoku, Mt. Garyuu	34°41′N, 132°11′E	1050	C	5801
	Chugoku, Hiroshima Pref., Geihoku, Mt. Garyuu	34°41′N, 132°11′E	1050	C	5817#
	Chugoku, Hiroshima Pref., Geihoku, Mt. Garyuu	34°41′N, 132°11′E	1050	C	5838
36	Chugoku, Yamaguchi Pref., Kano, Mt. Nagano	34°16′N, 131°52′E	1000	B	8242
	Chugoku, Yamaguchi Pref., Kano, Mt. Nagano	34°16′N, 131°52′E	1000	B	8277
	Chugoku, Yamaguchi Pref., Kano, Mt. Nagano	34°16′N, 131°52′E	1000	B	8286
37	Shikoku, Kagawa Pref., Shionoe, Mt. Ohtaki	34°07′N, 134°07′E	950	A′	4254
	Shikoku, Kagawa Pref., Shionoe, Mt. Ohtaki	34°07′N, 134°07′E	950	A′	4255
	Shikoku, Kagawa Pref., Shionoe, Mt. Ohtaki	34°07′N, 134°07′E	950	A′	4256
	Shikoku, Kagawa Pref., Shionoe, Mt. Ohtaki	34°07′N, 134°07′E	950	A′	4253
	Shikoku, Kagawa Pref., Shionoe, Mt. Ohtaki	34°07′N, 134°07′E	950	A′	4260
38	Shikoku, Tokushima Pref., Mima, Mt. Ryuuou	34°06′N, 134°02′E	1000	B	4306
39	Shikoku, Kochi Pref., Monobe, Mt. Shiraga	33°48′N, 133°58′E	1000	A′	4432
40	Shikoku, Kochi Pref., Hongawa, Mt. Kanpuu	33°48′N, 133°15′E	1400	B	4333
41	Shikoku, Ehime Pref., Shigenobu, Mt. Saragamine	33°43′N, 132°53′E	1100	B	4383
	Shikoku, Ehime Pref., Shigenobu, Mt. Saragamine	33°43′N, 132°53′E	1100	B	4385
	Shikoku, Ehime Pref., Shigenobu, Mt. Saragamine	33°43′N, 132°53′E	1100	B	4386
	Shikoku, Ehime Pref., Shigenobu, Mt. Saragamine	33°43′N, 132°53′E	1100	B	4394
	Shikoku, Ehime Pref., Shigenobu, Mt. Saragamine	33°43′N, 132°53′E	1100	B	4384
42	Shikoku, Kochi Pref., Higashitsuno, Mt. Irazu	33°26′N, 133°03′E	1300	B	492
	Shikoku, Kochi Pref., Higashitsuno, Mt. Irazu	33°26′N, 133°03′E	1300	B	496
43	Kyushu, Oita Pref., Takeda, Bogaturu	33°07′N, 131°15′E	1100	B	6272
	Kyushu, Oita Pref., Takeda, Bogaturu	33°07′N, 131°15′E	1100	B	6282
	Kyushu, Oita Pref., Takeda, Bogaturu	33°07′N, 131°15′E	1100	B	6278
	Kyushu, Oita Pref., Takeda, Bogaturu	33°07′N, 131°15′E	1100	B	6339
	Kyushu, Oita Pref., Takeda, Bogaturu	33°07′N, 131°15′E	1100	C	6402
44	Kyushu, Fukuoka Pref., Soeda, Mt. Hiko	33°28′N, 130°55′E	1000	B	5896#
	Kyushu, Fukuoka Pref., Soeda, Mt. Hiko	33°28′N, 130°55′E	1000	B	5921
	Kyushu, Fukuoka Pref., Soeda, Mt. Hiko	33°28′N, 130°55′E	1000	C	5919
	Kyushu, Fukuoka Pref., Soeda, Mt. Hiko	33°28′N, 130°55′E	1000	C	5929
45	Kyushu, Fukuoka Pref., Hukuoka, Mt. Sehuri	33°26′N, 130°22′E	1000	B	5879
	Kyushu, Fukuoka Pref., Hukuoka, Mt. Sehuri	33°26′N, 130°22′E	1000	C	5854
	Kyushu, Fukuoka Pref., Hukuoka, Mt. Sehuri	33°26′N, 130°22′E	1000	C	5868
	Kyushu, Fukuoka Pref., Hukuoka, Mt. Sehuri	33°26′N, 130°22′E	1000	C	5881
	Kyushu, Fukuoka Pref., Hukuoka, Mt. Sehuri	33°26′N, 130°22′E	1000	C	5884
46	Kyushu, Miyazaki Pref., Shiiba, The Ookouchi Pass	32°23′N, 131°11′E	1100	B	7521
47	Kyushu, Kumamoto Pref., Izumi, Momiki	32°30′N, 130°58′E	850	B	6785
	Kyushu, Kumamoto Pref., Izumi, Momiki	32°30′N, 130°58′E	850	B	6822
	Kyushu, Kumamoto Pref., Izumi, Momiki	32°30′N, 130°58′E	850	B	6867
	Kyushu, Kumamoto Pref., Izumi, Momiki	32°30′N, 130°58′E	850	B	6890
	Kyushu, Kumamoto Pref., Izumi, Momiki	32°30′N, 130°58′E	850	C	6760
48	Kyushu, Kumamoto Pref., Mizukami, Mt. Ichihusa	32°18′N, 131°05′E	1550	B	5991
49	Kyushu, Kagoshima Pref., Miyanojyo, Mt. Shibi	31°58′N, 130°22′E	1050	B	7124
	Kyushu, Kagoshima Pref., Miyanojyo, Mt. Shibi	31°58′N, 130°22′E	1050	B	7098
	Kyushu, Kagoshima Pref., Miyanojyo, Mt. Shibi	31°58′N, 130°22′E	1050	B	7182

Species and site number	Locality	Coordinates	Altitude (m)	Haplotype	Voucher specimen number
	Kyushu, Kagoshima Pref., Miyanojyo, Mt. Shibi	31°58′N, 130°22′E	1050	B	7178
Carpinus cordata Blume					
	Tohoku, Aomori Pref., Towadako, along the Ouse River	40°33′N, 140°56′E	500		2012#
Carpinus laxiflora (Siebold & Zucc.) Blume					
	Kinki, Nara Pref., Kurotaki, Mt. Yosuniwa	34°19′N, 135°54′E	1150		8631#

All samples and voucher specimens were collected by T. Iwasaki. #DNA samples were used for interspecific haplotype network analysis among four *Carpinus* species. *Only one voucher was collected for several samples.